




Multicenter Evaluation of the Portrait Staph ID/R Blood Culture Panel for Rapid Identification of Staphylococci and Detection of the *mecA* Gene

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ABSTRACT Bloodstream infections are a leading cause of morbidity and mortality in the United States and are associated with increased health care costs. We evaluated the Portrait Staph ID/R blood culture panel (BCP) multiplex PCR assay (Great Basin Scientific, Salt Lake City, UT) for the rapid and simultaneous identification (ID) of *Staphylococcus aureus*, *Staphylococcus lugdunensis*, and *Staphylococcus* species to the genus level and the detection of the *mecA* gene directly from a positive blood culture bottle. A total of 765 Bactec bottles demonstrating Gram-positive cocci in singles or clusters were tested during the prospective trial at 3 clinical sites. The Portrait Staph ID/R BCP results were compared with results from conventional biochemical and cefoxitin disk methods performed at an independent laboratory. Discordant ID and *mecA* results were resolved by *rpoB* gene sequencing and *mecA* gene sequencing, respectively. A total of 658 *Staphylococcus* species isolates (*S. aureus*, 211 isolates; *S. lugdunensis*, 3 isolates; and *Staphylococcus* spp., 444 isolates) were recovered from monomicrobial and 33 polymicrobial blood cultures. After discrepant analysis, the overall ratios of Portrait Staph ID/R BCP positive percent agreement and negative percent agreement were 99.4%/99.9% for *Staphylococcus* ID and 99.7%/99.2% for *mecA* detection.

KEYWORDS PCR, *Staphylococcus*, blood culture, genotypic identification, *mecA* detection, rapid tests

Despite advances in medical practices, bloodstream infections (BSIs) remain a significant cause of death in the United States. In the United States, sepsis is the leading cause of death among critically ill patients in noncoronary intensive care units (ICUs) and is the 10th leading cause of death overall, with the cost of care exceeding \$17 billion annually (1). Gram-positive organisms as a cause of sepsis are now more common than Gram-negative infections (2). The staphylococci are a major cause of hospital- and community-acquired infections, leading to serious infections associated with significant rates of morbidity and mortality (3). A study that included 49 U.S. hospitals revealed that the incidence rates and distribution of monomicrobial BSIs for coagulase-negative staphylococci (CoNS) and *Staphylococcus aureus* comprised approximately one-third and one-fifth of the total cases, respectively (4). In addition, the mortality rates for infections by these two pathogens totaled approximately 20% and 25%, respectively (4).

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TABLE 1 Summary of the clinical performance of Portrait Staph ID/R BCP versus conventional methods for the identification of *Staphylococcus* species in 765 prospective blood cultures^a

Organism(s) identified (all sites combined)	% Agreement			
	TP/TP + FN	PPA (95% CI)	TN/TN + FP	NPA (95% CI)
<i>S. aureus</i>	211/214	98.6 (96.0–99.5)	549/551	99.6 (98.7–99.9)
<i>S. lugdunensis</i>	3/3	100 (43.9–100)	761/762	99.9 (99.3–99.9)
<i>Staphylococcus</i> species other than <i>S. aureus</i> or <i>S. lugdunensis</i>	444/449	98.9 (97.4–99.5)	311/313	99.4 (97.7–99.9)

^aTP, true positive; TN, true negative; FP, false positive; FN, false negative; PPA, positive percent agreement; NPA, negative percent agreement; CI, confidence interval.

Currently, blood cultures continue to be the standard method for diagnosing BSIs (5). This conventional method requires additional testing *in vitro* to identify the organism and obtain an antimicrobial susceptibility profile. Thus, results will not be available to the physician until 2 to 3 days from the moment the blood culture is identified as positive. The availability of rapid detection of such pathogens is necessary to decrease the time between detection and treatment. This is of utmost importance, as it has been shown that mortality increases with each day of inappropriate treatment (6).

The Portrait Staph ID/R blood culture panel (BCP) is a qualitative, multiplex, nucleic acid-based assay that simultaneously identifies *Staphylococcus aureus*, *Staphylococcus lugdunensis*, and various *Staphylococcus* species to the genus level and detects the *mecA* gene, the primary determinant of methicillin resistance, directly from positive blood cultures. The test utilizes automated hot-start-enabled PCR for amplification of specific DNA targets detected by hybridization probes immobilized on a silicon chip surface. The Great Basin PA500 Portrait system is a fully automated system that includes the Portrait Analyzer, single-use Staph ID/R BCP cartridges, and the Portrait data analysis software. The Portrait system is designed to perform automated sample preparation, PCR, and optimal chip-based detection with integrated data analysis in 110 min.

The purpose of this multicenter study was to assess the performance of the Portrait Staph ID/R BCP compared to conventional methods.

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RESULTS

The clinical performances of Portrait Staph ID/R BCP and of conventional methods for the identification of *Staphylococcus* species and detection of *mecA* are summarized in Tables 1 and 2, respectively. A total of 765 mono- and polymicrobial prospective blood cultures meeting the criteria for inclusion were included in the clinical trial. The Portrait Staph ID/R BCP identified *S. aureus* (211 isolates), *S. lugdunensis* (3 isolates), and

TABLE 2 Summary of the clinical performance of Portrait Staph ID/R BCP versus cefoxitin disk diffusion test method for the detection of methicillin resistance in 762 prospective blood cultures^a

Result, all sites combined, for detection of methicillin resistance	% Agreement			
	TP/TP + FN	PPA (95% CI)	TN/TN + FP	NPA (95% CI)
<i>S. aureus</i>	72/75	96.0 (86.6–97.8)	682/687	99.3 (98.3–99.7)
<i>S. lugdunensis</i>	0/0	NA	762/762	100 (99.5–100)
<i>Staphylococcus</i> spp. other than <i>S. aureus</i> or <i>S. lugdunensis</i>	247/263	93.9 (90.3–96.2)	481/499	96.4 (94.4–97.7)

^aThree culture isolates of *Staphylococcus* failed to grow on Mueller-Hinton agar for cefoxitin testing. TP, true positive; TN, true negative; FP, false positive; FN, false negative; PPA, positive percent agreement; NPA, negative percent agreement; CI, confidence interval. NA, not applicable.

TABLE 3 *Staphylococcus* species identification discordant sequencing results for *rpoB* (all sites combined)

Portrait Staph ID/R BCP result	Discordant result (n) ^b	BD Phoenix result	<i>rpoB</i> sequencing result
<i>S. aureus</i> in mixed Staph ^a infection (not <i>S. lugdunensis</i>)	FP for mixed (1)	<i>S. aureus</i>	<i>S. aureus</i>
<i>S. aureus</i> in mixed Staph infection (not <i>S. lugdunensis</i>)	FP (1)	<i>Staphylococcus epidermidis</i>	<i>S. epidermidis</i>
<i>S. aureus</i> in mixed Staph infection (not <i>S. lugdunensis</i>)	FP (1)	<i>Staphylococcus hominis</i>	<i>S. hominis</i>
<i>S. lugdunensis</i> in mixed Staph infection (not <i>S. aureus</i>)	FP (1)	<i>S. epidermidis</i>	<i>S. epidermidis</i>
Staph species other than <i>S. aureus</i> or <i>S. lugdunensis</i>	FP (2)	<i>Corynebacterium jeikeium</i>	<i>Staphylococcus pettenkoferi</i>
Staph species other than <i>S. aureus</i> or <i>S. lugdunensis</i>	FP (1)	<i>Rothia mucilaginosa</i>	<i>S. epidermidis</i>
Staph species other than <i>S. aureus</i> or <i>S. lugdunensis</i>	FP (1)	<i>R. mucilaginosa</i> / <i>Streptococcus gordonii</i>	<i>S. hominis</i> / <i>S. epidermidis</i>
Staph species other than <i>S. aureus</i> or <i>S. lugdunensis</i>	FN (2)	<i>S. aureus</i>	<i>S. epidermidis</i>
Staph species other than <i>S. aureus</i> or <i>S. lugdunensis</i>	FN (1)	<i>S. aureus</i> / <i>S. epidermidis</i> / <i>S. hominis</i>	<i>S. epidermidis</i>
Negative	FN (1)	<i>S. epidermidis</i>	<i>S. epidermidis</i>
Negative	FN (1)	<i>S. hominis</i>	<i>S. epidermidis</i>

^amixed Staph, mixed *Staphylococcus* spp.^bFor a total of 13 isolates; FP, false positive; FN, false negative.

Staphylococcus species other than *S. aureus* or *S. lugdunensis* (444 isolates) with positive percent agreement (PPA) of 98.6%, 100%, and 98.9% and negative percent agreement (NPA) of 99.6%, 99.9%, and 99.4%, respectively (Table 1). Overall, there were 13 discordant species in identification with the Staph ID/R BCP and the BD Phoenix conventional method (Table 3). Of the 13 discordant results called, 8 were false positive (FP) and 5 were false negative (FN). When the 13 discordant specimens were tested by *rpoB* sequencing, 7 results were in agreement with Portrait Staph ID/R BCP (Table 3). After discrepant analysis, the overall Portrait Staph ID/R BCP PPA and NPA for *Staphylococcus* ID increased from 98.8% and 99.7%, respectively, to 99.4% and 99.9%.

The Portrait Staph ID/R BCP detected *mecA* in *S. aureus* (69 isolates) and *Staphylococcus* species other than *S. aureus* or *S. lugdunensis* (243 isolates) with PPA of 96.0% and 93.9% and NPA of 99.3% and 96.4%, respectively (Table 2). Three culture isolates of *Staphylococcus* failed to grow on Mueller-Hinton agar for cefoxitin, resulting in 762 blood cultures tested. Overall there were 42 discordant *mecA* detection results compared to cefoxitin disk diffusion (23 FP and 19 FN), irrespective of species identification. For 24/41 discordant specimens, *mecA* sequencing results were concordant with the Portrait Staph ID/R BCP. One specimen was not subjected to discordant analysis. The remaining 16/41 specimens consisted of 15 FP and 1 FN *mecA* sequencing results.

A total of 2.3% (18/765) polymicrobial blood culture specimens were examined by Portrait Staph ID/R BCP and conventional methods (Table 4). Conventional cultures accounted for 1.7% (13/765) of the polymicrobial specimens during the study, with 33 isolates recovered. The Portrait Staph ID/R BCP "mixed Staph" infections accounted for 0.5% (4/765) of the polymicrobial specimens, and 1 of 765 specimens (0.1%) was polymicrobial for both Portrait Staph ID/R BCP and conventional cultures. When specimens with conventional polymicrobial results were compared to the Portrait Staph ID/R BCP, a single FN result for *S. aureus* and two FN *mecA* results were obtained by Staph ID/R BCP. In four specimens with Portrait Staph ID/R BCP in mixed infections, three were FP for *S. aureus* compared to culture and one specimen was correct for *S. aureus* but FP for mixed *Staphylococcus* infection and *mecA*. Polymicrobial results by both Portrait Staph ID/R BCP and conventional methods displayed complete agreement in one specimen.

The overall initial invalid result rate in this prospective study was 1.39%. The overall aborted run or "test incomplete" rate was 3.30%. Valid results were achieved after retesting all invalid-result and test-incomplete runs.

TABLE 4 Performance of Portrait Staph ID/R BCP and/or conventional methods for polymicrobial specimens^a

				Discrepant result	
Staph ID/R species ID, <i>mecA</i> results	Conventional method species ID, cefoxitin susceptibility results			Species ID	<i>mecA</i>
Polymicrobial specimens for conventional methods					
Staph species other than <i>S. aureus</i> or <i>S. lugdunensis</i> , <i>mecA</i> absent	<i>S. aureus</i> , sensitive	<i>S. epidermidis</i> , not tested	<i>S. hominis</i> , not tested	FN <i>S. aureus</i>	
Staph species other than <i>S. aureus</i> or <i>S. lugdunensis</i> , <i>mecA</i> absent	<i>S. epidermidis</i> , sensitive	<i>S. epidermidis</i> , resistant			FN
Staph species other than <i>S. aureus</i> or <i>S. lugdunensis</i> , <i>mecA</i> absent	<i>S. capitis</i> , sensitive	<i>S. pettenkoferi</i> , resistant			FN
Staph species other than <i>S. aureus</i> or <i>S. lugdunensis</i> , <i>mecA</i> absent	<i>S. capitis</i> , sensitive	<i>S. epidermidis</i> , no growth			
Staph species other than <i>S. aureus</i> or <i>S. lugdunensis</i> , <i>mecA</i> absent	<i>S. epidermidis</i> , sensitive	<i>S. haemolyticus</i> , sensitive			
Staph species other than <i>S. aureus</i> or <i>S. lugdunensis</i> , <i>mecA</i> absent	<i>S. epidermidis</i> , sensitive	<i>S. capitis</i> , sensitive			
Staph species other than <i>S. aureus</i> or <i>S. lugdunensis</i> , <i>mecA</i> absent	<i>S. epidermidis</i> , sensitive	<i>S. warneri</i> , sensitive			
Staph species other than <i>S. aureus</i> or <i>S. lugdunensis</i> , <i>mecA</i> absent	<i>S. hominis</i> , sensitive	<i>S. capitis</i> , sensitive			
Staph species other than <i>S. aureus</i> or <i>S. lugdunensis</i> , <i>mecA</i> present	<i>S. capitis</i> , sensitive	<i>S. epidermidis</i> , resistant			
Staph species other than <i>S. aureus</i> or <i>S. lugdunensis</i> , <i>mecA</i> present	<i>S. hominis</i> , resistant	<i>S. epidermidis</i> , resistant			
Staph species other than <i>S. aureus</i> or <i>S. lugdunensis</i> , <i>mecA</i> present	<i>S. hominis</i> , resistant	<i>S. epidermidis</i> , resistant			
Staph species other than <i>S. aureus</i> or <i>S. lugdunensis</i> , <i>mecA</i> present	<i>S. hominis</i> , resistant	<i>S. capitis</i> , sensitive			
Staph species other than <i>S. aureus</i> or <i>S. lugdunensis</i> , <i>mecA</i> present	<i>S. epidermidis</i> , resistant	<i>S. haemolyticus</i> , sensitive			
Polymicrobial specimens for Staph ID/R BCP in mixed <i>Staphylococcus</i> infections					
<i>S. aureus</i> in mixed Staph infection (not <i>S. lugdunensis</i>), <i>mecA</i> absent	<i>S. epidermidis</i> , sensitive			FP <i>S. aureus</i>	
<i>S. aureus</i> in mixed Staph infection (not <i>S. lugdunensis</i>), <i>mecA</i> present	<i>S. hominis</i> , resistant			FP <i>S. aureus</i>	
<i>S. aureus</i> in mixed Staph infection (not <i>S. lugdunensis</i>), <i>mecA</i> present	<i>S. aureus</i> , sensitive			FP mixed	FP
<i>S. aureus</i> in mixed Staph infection (not <i>S. lugdunensis</i>), <i>mecA</i> present	<i>S. epidermidis</i> , resistant			FP <i>S. aureus</i>	
Polymicrobial specimens for both Staph ID/R BCP and conventional methods					
<i>S. aureus</i> in mixed Staph infection (not <i>S. lugdunensis</i>), <i>mecA</i> present	<i>S. aureus</i> , sensitive	<i>S. epidermidis</i> , resistant			

^aFN, false negative; FP, false positive; Staph, *Staphylococcus*.

Various Bactec bottle types were evaluated in this study. The most frequent Bactec bottle type used in this study was Bactec Plus Aerobic/F ($n = 333$), followed by Bactec Standard/10 Aerobic/F ($n = 182$), with the Bactec Lytic/10 Anaerobic/F medium ($n = 77$), Bactec Plus Anaerobic/F medium ($n = 11$), and Bactec Standard/10 Anaerobic/F medium ($n = 90$) being used less. The Bactec Peds Plus/F medium (BD) was also tested ($n = 62$). The blood culture bottle types used in the study did not display any apparent performance difference (data not shown).

DISCUSSION

Staphylococcal species are the leading cause of BSIs worldwide, and up to 60% of all staphylococcal infections are methicillin resistant. *Staphylococcus aureus* is the most common pathogen found in positive blood cultures, with a high prevalence of methicillin-resistant *S. aureus* (MRSA). Patients with MRSA bacteremia have demonstrated a higher mortality rate than those with methicillin-susceptible *S. aureus* (MSSA) bacteremia (7). The CoNS are common and typically nonthreatening inhabitants of skin and mucus membranes. However, CoNS can cause human infections that characteris-

tically involve indwelling medical devices. Clinically significant bacteremia caused by CoNS remains problematic and difficult to interpret (8, 9). *Staphylococcus lugdunensis* is a species of CoNS that can cause severe and potentially fatal disease, including vascular catheter infections, bacteremia, and sepsis (10). Outcome studies have shown that a single *S. lugdunensis*-positive blood culture can represent a clinically significant bacteremia and should not be classified as a contaminant (11).

The high prevalence of MRSA combined with the slow turnaround time for organism identity and antibiotic resistance information has led physicians to use empirical therapy with agents such as vancomycin for suspected staphylococcal infections. It has been demonstrated elsewhere that up to one-third of all staphylococcal infections are treated suboptimally due to inappropriate empirical therapy. Schweizer et al. (12) demonstrated that patients with MSSA bacteremia had significantly lower mortality when treated with a more appropriate beta-lactam antimicrobial than when treated with vancomycin. Rapid identification of the causative agent and detection of resistance mechanisms, like the *mecA* gene in patients with BSIs, can provide the physician important information to guide appropriate treatment in a timely manner, leading to improved patient outcomes. Studies using a rapid molecular *S. aureus*/MRSA test from positive blood cultures have reduced the time of diagnosis from 24 to 48 h to 1 to 2 h following indication of a blood culture being Gram-positive cocci in singles or clusters (GPCC) positive, leading to improved patient care, antimicrobial management, length of patient stay, and hospital costs (6, 13, 14). These methods are most effective when an active antimicrobial stewardship program is available (15). The impact of rapid peptide nucleic acid fluorescence *in situ* hybridization (PNA FISH) testing demonstrated how excessive vancomycin usage could be prevented for the treatment of CoNS-contaminated blood cultures (16). In addition, early identification of CoNS in blood cultures, thus ruling out *S. aureus* or *S. lugdunensis*, could avoid unnecessary treatment and hospital stay for patients found to have a blood culture contaminant. The value of appropriate therapeutic decisions makes molecular diagnostic tests an attractive alternative to conventional blood culture methods that can take up to several days to complete.

The Portrait Staph ID/R system is an automated benchtop analyzer with low-cost, disposable cartridges for performing on-demand testing during any shift. The immediate benefit of the Portrait Staph ID/R system is the minimal sample handling (sample in/results out). The Portrait Staph ID/R system can be easily integrated into the normal laboratory workflow, providing decreased time to identification and resistance results. Invalid and "test incomplete" rates in this study were low, and these issues were resolved on retesting. In additional analytical studies, Portrait Staph ID/R was shown to have equivalent performance on Bactec with 6 bottle types, BacT/Alert with 5 bottle types, and 2 bottle types with Versa Trek blood culture systems (data not shown). The equivalence in performance across the three major blood culture systems including 13 different bottle types indicates a broad tolerance to medium types.

In this study, there were 42 discordant *mecA* results compared to the results obtained by testing of colonies isolated from the positive blood culture using cefoxitin disk diffusion as the comparator method. False-negative results ($n = 19$) could be explained by specific *mecI*-mediated repression of *mecA* transcription and penicillin binding protein 2a (PBP 2a) production and derepression of *mecA* following the induction by cefoxitin (17). However, only one unresolved falsely negative discordant *mecA* result (Portrait Staph ID/R BCP reported the presence of *Staphylococcus* species other than *S. aureus* or *S. lugdunensis*, *mecA* negative, in contrast to the conventional method result) occurred in blood cultures in which a single organism was detected. This suggests that the Portrait Staph ID/R assay was highly accurate for detecting the presence of the *mecA* gene. Interestingly, 15/23 false-positive results were unresolved by testing of isolates by PCR/*mecA* gene sequencing, which may point out a limitation of the study design. In a study evaluating the FilmArray BCID panel (18), overall positive and negative predictive agreement was determined in comparison to PCR/sequencing of cultured isolates (99.2% and 86.6%, respectively, for all *mecA* results combined) and PCR/sequencing directly from blood cultures (98.4% and 98.0%, respectively). The lower

negative predictive agreement obtained when performing PCR/sequencing on isolates than directly from positive blood culture indicates the potential for the presence of multiple indistinguishable organisms in the blood culture broth with different resistance profiles.

In this study, polymicrobial blood cultures were low, accounting for 1.7% (13/765) by the reference culture method and 0.5% (4/765) by the Portrait Staph ID/R BCP assay, although the several cases of unresolved false-positive *mecA* results suggest an under-detection of poly-CoNS samples. While the Portrait Staph ID/R BCP can detect mixtures of *S. aureus* with CoNS, it cannot detect mixtures containing multiple CoNS species other than *S. lugdunensis*. Polymicrobial sepsis is considered uncommon, occurring at a rate of 4.7% in one series of septic episodes (19). The impact of detecting multiple CoNS in blood cultures in these cases would be minimal since they would be classified as probable contaminants.

One limitation of the Portrait Staph ID/R system is that it targets detection of only staphylococci and *mecA*. Inclusion of other species-specific targets such as the streptococci and enterococci as well as resistance markers *vanA* and *vanB* would enhance the utility of the Portrait Staph ID/R system for Gram-positive organisms. However, the typical microbiology laboratory can readily detect the phenotype of Gram-positive cocci in clusters. With staphylococci being the most prevalent cause of bloodstream infection seen and the clinical utility of these tests resulting in lower treatment costs and lengths of stay, a more focused test like this one may have value in the laboratory, especially at its significantly lower price (\$45) than that of a broader panel, priced at \$75 to \$129 (20, 21). Another potential limitation of PCR diagnostic testing is the emergence of *mecC* MRSA (22). Similar to *mecA*, *mecC* is carried on the staphylococcal chromosomal cassette *mec* gene that codes for a PBP 2a, which mediates resistance to beta-lactam antibiotics. Although *mecC* MRSA is currently extremely rare, the possibility exists that *mecA* MRSA assays will fail to detect isolates that contain *mecC*. Finally, the Portrait Staph ID/R system does not replace conventional identification and antimicrobial susceptibility testing. Supplemental testing contributes to additional expense to the laboratory budget.

In conclusion, the performance of the Portrait Staph ID/R BCP in this study was found to be highly favorable compared to conventional methods. The Portrait Staph ID/R BCP can identify the most clinically relevant *Staphylococcus* species that are increasingly associated with true infections. The decreased time to results has benefits of improved patient outcomes and promotes antimicrobial stewardship.

MATERIALS AND METHODS

Blood culture specimens. The performance of the FDA-approved Portrait Staph ID/R BCP assay was determined prospectively at three geographically diverse U.S. clinical sites. Blood cultures were collected in BD Bactec aerobic (Plus Aerobic/F, Peds Plus/F, and Standard/10 Aerobic/F) and anaerobic (Plus Anaerobic/F, Lytic/10 Anaerobic/F, and Standard/10 Anaerobic/F) blood culture bottles as part of the routine standard of care at each facility and incubated on a Bactec FX Instrumented Blood Culture System (BD Diagnostics, Sparks, MD). Positive Bactec blood culture bottles that contained Gram-positive cocci in singles or clusters (GPCC) from unique patients were enrolled between December 2014 and May 2015 at Indiana University Health Pathology Laboratory, University of New Mexico/Tricore Laboratories, and University of Utah, Primary Children's Medical Center clinical microbiology laboratories. Positive blood cultures were processed according to routine laboratory procedures at each site. After completion of clinical processing, 1-ml aliquots of remnant positive blood culture specimens were aseptically removed from the blood bottle. Specimens were collected within 8 h of positive detection and stored at room temperature for up to 18 h or refrigerated at 2 to 8°C for up to 72 h before Portrait testing. Two 1-ml aliquots were frozen at -70°C and sent to the Medical College of Wisconsin laboratory for discordant testing. Specimens were used in accordance with procedures approved by the Institutional Review Board at each test site with a waiver for informed consent.

Portrait Staph ID/R BCP assay. The Portrait Staph ID/R BCP assay was performed according to the manufacturer's instructions. Briefly, the Portrait Staph ID/R BCP assay is built into an injection-molded card. Reagents are lyophilized and placed in blister packs. The operator inserts a 50- μ l aliquot of positive blood culture broth into the sample port, inserts the card into the PA500 Portrait Analyzer, and presses the start key to initiate the test. The Portrait system is designed to perform automated sample preparation, hot-start PCR amplification of specific *Staphylococcal* DNA detected by hybridization probes immobilized on a silicon chip. Result options are reported as follows: *Staphylococcus aureus* \pm *mecA*; *Staphylococcus lugdunensis* \pm *mecA*; *Staphylococcus* species other than *S. aureus* or *S. lugdunensis* \pm *mecA*; *S. aureus* in mixed *Staphylococcus* infection (not *S. lugdunensis*) \pm *mecA*; *S. lugdunensis* in mixed

Staphylococcus infection (not *S. aureus*) \pm *mecA*; *S. aureus*, *S. lugdunensis* \pm *mecA*; *S. aureus*, *S. lugdunensis* in mixed infection \pm *mecA*; Negative—no *Staphylococcus* species detected; or Invalid—internal control failed. The assay is completed in 110 min. Any “No Calls” (invalid or aborted run) results were retested.

Conventional procedures. An aliquot of positive blood was inoculated onto 5% sheep blood agar and incubated for 48 to 72 h at 35 to 37°C. *Staphylococcus* isolates were identified using a combination of biochemical tests catalase, coagulase, and BD Phoenix System (Sparks, MD). Detection of methicillin resistance was performed using the cefoxitin disk diffusion test according to CLSI guidelines (23).

Data analysis. The performance of the Portrait Staph ID/R BCP assay was compared to a biochemical method for species identification and to cefoxitin disk diffusion for detection of methicillin resistance. Discordant identification results were arbitrated by *rpoB* gene sequencing (24). Discordant resistance detection results were arbitrated by sequencing of the *mecA* gene by PCR from single bacterial colonies. The positive percent agreement (PPA) and negative percent agreement (NPA) of the Portrait Staph ID/R BCP assay compared to conventional methods were calculated. The 95% confidence intervals (CI) were determined for performance estimates using results across all three geographical sites.

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